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GENETICS AND PLANT BREEDING

Detection of genetic diversity in *Lathyrus sativus* L. using RAPD marker system

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Abstract

Lathyrus sativus L. (Grasspea) is a protein rich pulse crop. Enough information about genetic diversity of Lathyrus germplasm is needed to improve this crop. Molecular characterization is one of the tools to identify the hidden genetic diversity. The present study was undertaken to reveal the genetic diversity in a set of 20 grasspea genotypes using RAPD (Randomly Amplified Polymorphic DNA) marker system. For the 15 primers, various efficiency parameters that include Polymorphism Information Content (PIC), Marker Index (MI), Resolving Power (RP) and Diversity Index (DI) were studied. Finally Principal coordinate analysis (PCoA) and dendrogram was perfomed to find out the genetic diversity among the germplasm. The screened RAPD markers will facilitate further studies in population genetics and utilization of this crop.

Highlights

- Selected RAPD markers are highly informative for these grasspea genotypes.
- Four efficiency parameters of RAPD marker system were studied.
- Three clusters were obtained on the basis of RAPD profiling.

Keywords: Genetic diversity, grasspea, randomly amplified polymorphic DNA

Grasspea (*Lathyrus sativus* L.), popularly known as khesari, is a very hardy pulse crop proficient of growing in drought condition. Total cultivated area for this crop is 6.0 lakh hector in India. Madhya Pradesh, Bihar, West Bengal, Maharashtra and parts of eastern Uttar Pradesh are the most extensively cultivated area. It is mostly grown in the month of October- November as a rabi crop and harvested in late February or early March. For the presence of BOAA (β-N-oxalyl-amino-L-alanine), a neurotoxic compound (causes lathyrism) it has limited uses for human and animal consumption. This toxin content could be significantly reduced by careful selection and through hybridization between low and high toxin varieties (Khan *et al.,* 2001; Brahim *et al.,* 2011). For this, extensive knowledge about genetic diversity of the crop is essential. It can be obtained from pedigree analysis, morphological study as well as molecular study. In breeding programs of conservation genetics, assessing the degree of genetic variation and partitioning total genetic variation within and between populations in the cultivated accessions are important (Nybom and Bartish, 2000). In addition, understanding of the population genetic structure of a species is indispensable to make valid biological elucidation about its breeding system and reproductive biology (Smartt 1981; Bussell 1999). For detection of genetic diversity in different crops RAPD marker is being used as an efficient technique (Madhuri *et al.,* 2012; Chakraborty *et al.,* 2013; Gami

Table 1. List of fifteen RAPD primers and their efficiency parameters

Sl No.	Primer Id	Sequence (5'-3')	PIC	NPB	MI	RP	DI
1	OPA-01	CAGGCCCTTC	0.30	5.00	1.50	2.4	0.23
2	OPA-04	AATCGGGCTG	0.30	5.00	1.49	2	0.57
3	OPE-01	CCCAAGGTCC	0.43	3.00	1.30	2.5	0.20
4	OPE-06	AAGACCCCTC	0.30	7.00	2.12	2.9	0.46
5	OPE-12	TTATCGCCCC	0.34	4.00	1.35	2.2	0.33
6	OPE-19	ACGGCGTATG	0.45	6.00	2.69	4.8	0.28
7	OPF-06	GGGAATTCGG	0.36	6.00	2.15	3.5	0.58
8	OPF-16	GGAGTACTGG	0.44	6.00	2.61	4.2	0.25
9	OPJ-09	TGAGCCTCAC	0.42	2.00	0.83	1.2	0.90
10	OPAC-12	GGCGAGTGTG	0.28	4.00	1.11	1.8	0.54
11	OPAO-08	ACTGGCTCTC	0.40	2.00	0.80	1.2	0.50
12	OPAR-01	CCATTCCGAG	0.41	4.00	1.64	2.5	0.48
13	OPA-02	TGCCGAGCTG	0.47	6.00	2.82	4.3	0.25
14	OPA-09	GGGTAACGCC	0.43	3.00	1.29	2.1	0.43
15	OPA-20	GTTGCGATCC	0.26	4.00	1.02	1.5	0.68

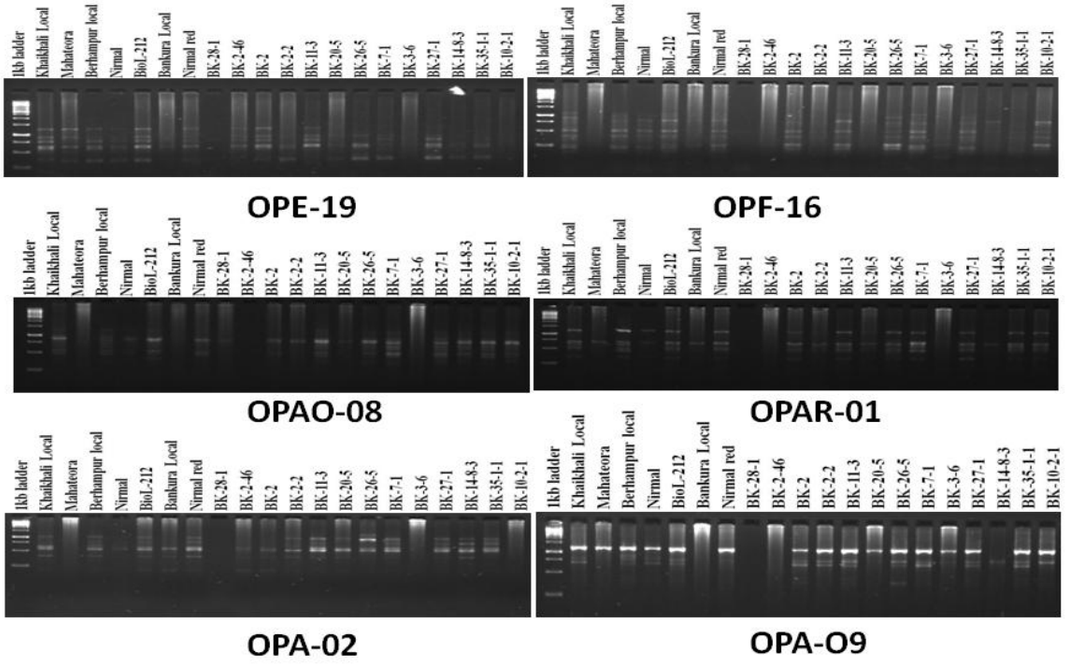

Figure 1. RAPD pattern of six primers produced from DNA amplification of the twenty grasspea genotypes

et al., 2013). In this experiment, RAPD marker system is used to investigate the extent and distribution of genetic diversity in twenty grasspea genotypes collected from different parts of India.

Materials and Methods

Twenty grasspea genotypes obtained from Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya, West Bengal were used for molecular study. The grasspea genotypes were Kaikhali local, Mahateora, Berhampur local, Nirmal, BioL-212, Bankura local, Nirmal Red, BK-28-1, BK-2-46, BK-2, BK-2-2, BK-11-3, BK-20-5, BK-26-5, BK-7-1,BK-3-6, BK-27-1, BK-14-8-3, BK-35-1-1 and BK-10-2-1. The experiment was conducted in September, 2013, plants were grown in polyhouse and DNA was isolated from each genotype. Total genomic DNA was extracted from the young leaves following Mandal *et al*. (2014) method.

In total, 15 RAPD primers were used (Table 1). PCR amplifications were performed in 25 µl reaction mixtures containing 1 U *Taq* polymerase (Biotools), 2.5 µl 10X PCR buffer, 1 µl 2.5 mM of each dNTPs 2 µl10 µM of primer and approximately 10 ng genomic DNA. Cycling conditions were 5 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45 s annealing at 42°C, a 1 min extension at 72°C, and a final extension step of 2 min at 72°C. Products were resolved on a 1 % agarose gel and visualized by Ethidium Bromide (Figure 1).

Product sizes were determined by comparison to a 1 kb DNA ladder (Biotools). To determine the most informative primer, following parameters were calculated *viz*. Polymorphism Information Content (PIC), Marker Index (MI), Resolving Power (RP), and Diversity Index (DI) as described by Quintero *et al*. (2011). The PIC value for each RAPD primer was calculated according to the formula $PIC_i = 2f_i (1-f_i)$, in which f_i is the frequency of the marker fragments that were present, and $1-f_i$ is the frequency of the marker fragments that were absent. PIC was averaged over all fragments for each primer. The marker index (MI) was calculated according to the formula MI = DI × EMR, in which EMR is the effective multiplex ratio, defined as the product of the total number of loci per fragments per primer (n) and the fraction of polymorphic loci fragments (β) (EMR = nβ). The resolving power (RP) of each primer was calculated as $RP = \Sigma I_b$, in which I_b represents fragment informativeness. The I_b can be represented on a 0 /1 scale using the formula $I_b = 1- [2 \times (0.5 - P)]$, in which P is the proportion of the 12 accessions that contain the fragment. The diversity index, which indicates the genetic diversity of the germplasm, was calculated using the formula $DI = 1 - 1/L \Sigma Pi^2$, in which Pi is the allele frequency (each individual allele is considered a unique fragment amplification) and L is the number of loci. Each primer received a score (1 for presence and 0 for absence of bands in each accession), and a binary matrix was generated (Saini *et al*., 2013). A dendrogram was constructed using the Dice similarity coefficient, the unweighted pair group method with arithmetic average (UPGMA) and the jackknife method for corroboration. All calculations were performed using NTSYS PC version 2.2 (Rohlf, 2012) and moreover, a principal coordinate analysis (PCoA), using the DSC (Nei and Li, 1979), was conducted with DARwin version 5.0.

Results and Discussion

RAPD marker system is efficient and cost-effective to generate molecular data and have been used auspiciously in various phylogenetic studies (Abo-elwafa *et al*., 1995; Sharma *et al*., 1995; Friesen *et al*., 1997; Wolff and Morgan, 1998; Fernandes *et al*., 2011). The variable response of grasspea genotypes were characterized by using the 15 RAPD primers. The total numbers of amplification products generated were 67 and all the amplicons were found to be polymorphic. The number of amplification products in 15 arbitrary primers ranged from 2 to 6 (Table 1). The molecular weight of the generated bands in the present study ranged from 100 bp to1.5 kb. Maximum number of fragments (6) were amplified by OPE-19, OPF-06, OPF-16 and OPJ09. Minimum numbers (2) of RAPD products were obtained with OPJ-09 and OPAO-08. Moreover, eight tested primer (OPE-01, OPE-19, OPF-16, OPJ-09, OPAO-08, OPAR-01, OPA-02 and OPA-09) had PIC values greater than 0.40. The high average genetic diversity observed in the present study is 0.90 (OPJ-09). The OPA-02 marker was highly polymorphic and had a high (0.47) PIC

value (Table-1). The estimates of RP were found to be the highest for the primer OPE-19 (4.8), followed by OPA-02 (4.3), OPF-16 (4.2) and was lowest (1.2) for the primer OPAO-08 and OPJ-09. The maximum MI was observed for the primer OPA-02 (2.82) followed by the primer OPE-19 (2.69) and OPF-16 (2.61). The minimum MI was observed again for the primer OPAO-08 (0.80).

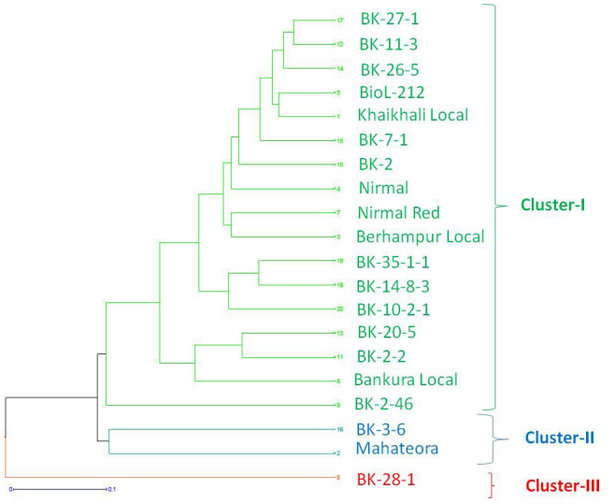

Figure 2. UPGMA cluster analysis of RAPD data generated fifteen primers for twenty genotypes of grasspea depicting patterns of genetic diversity

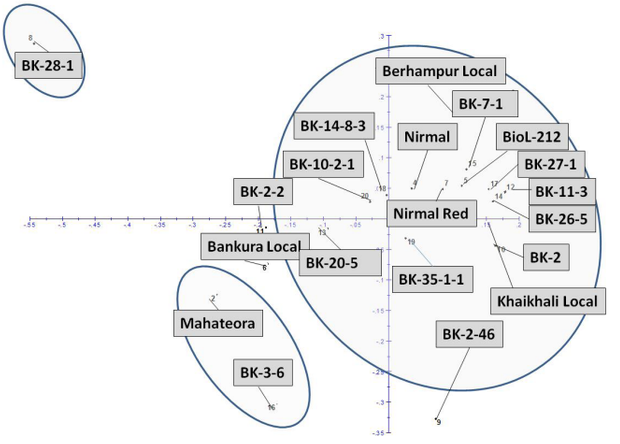

Figure 3. Two-dimensional representation of twenty genotypes of grasspea according to PCoA analysis.

The RAPD cluster analysis showed three major clusters namely cluster-I, cluster-II and cluster–III comprising of 17, 2 and 1 genotypes respectively. Cluster-I includes seventeen genotypes *viz.* Kaikhali local, Berhampur local, Nirmal, BioL-212, Bankura local, Nirmal Red, BK-2-46, BK-2, BK-2-2, BK-11-3, BK-20-5, BK-26-5, BK-7-1, BK-27-1, BK-14-8-3, BK-35-1-1 and BK-10-2-1. Cluster –II also includes two genotypes Mahateora and BK-3-6. Cluster-III includes the only one genotype BK-28-1 (Figure 2).

In order to validate efficiency of primers in distinguishing genetic diversity, PCoA was performed, which determined that the first three principal coordinates accounted for 68.48% of total variation (Figure 3). The RAPD marker system depicted the polymorphism may be due to a single-base change in DNA level. In this study, RAPD generated a higher number of bands because RAPDs are arbitrary in nature and can anneal randomly in the genome. The only difference at molecular level may be due to point mutation or natural recombination during their long span of cultivation in different areas which differ on the basis of soil types, climatic conditions and cultivation practices. The recombination event in support of adaptation to the environment leads to the creation of distinct genotypes. Molecular markers, unlike morpho-agronomic traits, are not influenced by environmental conditions and, therefore, are more reliable tools not only to characterize genotypes, but also to measure genetic relationship more precisely. Similar work has been done by the Norsati *et al.*, (2012). Present study established the utility of DNA finger-printing in genotypes using RAPD marker which revealed the presence of genetic diversity among the genotypes studied. RAPDs proved to be a high resolution technique for the detection of genetic variation among and within populations of *L. sativus*, supported by Sedehi *et al.*, (2008).

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